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Revisiting streamside trees that do not use stream water: can the two water worlds hypothesis and snowpack isotopic effects explain a missing water source?

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Keywords
Acer negundo, Acer grandidentatum, riparian, water, snow, groundwater

Disciplines
Ecology and Evolutionary Biology | Geochemistry | Hydrology | Soil Science

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Abstract

We revisit a classic ecohydrological study that showed streamside riparian trees in a semi-arid mountain catchment did not use perennial stream water. The original study suggested that mature individuals of *Acer negundo*, *A. grandidentatum*, and other species were dependent on water from "deeper strata", possibly groundwater. We used a dual stable isotope approach ($\delta^{18}O$ and $\delta^{2}H$) to further examine the water sources of these trees. We tested the hypothesis that groundwater was the main tree water source, but found that neither groundwater nor stream water matched the isotope composition of xylem water during two growing seasons. Soil water (0 – 1 m depth) was closest to and periodically overlapped with xylem water isotope composition, but overall, xylem water was isotopically enriched compared to all measured water sources. The "two water worlds" hypothesis postulates that soil water comprises isotopically distinct mobile and less-mobile pools that do not mix, potentially explaining this disparity. We further hypothesized that isotopic effects during snowpack metamorphosis impart a distinct isotope signature to the less-mobile soil water that supplies summer transpiration. Depth trends in water isotopes following snowmelt were consistent with the two water worlds hypothesis, but snow metamorphic isotope effects could not explain the highly-enriched xylem water. Thus, the dual isotope approach did not unambiguously determine the water source(s) of these riparian trees. Further exploration of physical, geochemical, and biological mechanisms of water isotope fractionation and partitioning are necessary to resolve these data, highlighting critical challenges in the isotopic determination of plant water sources.

Short title: Revisiting streamside trees that do not use stream water

Keywords *Acer negundo*, *Acer grandidentatum*, riparian, water, snow, groundwater

Introduction

Investigations of plant water use are fundamental to terrestrial ecohydrology. Since isotopes of precipitation vary across the landscape via well-understood processes (Gat 1996), there is a wealth of information about the hydrologic cycle contained in the stable isotopes of water. Most plants do not alter the isotope composition of water during uptake by roots or transport through xylem (White et al. 1985, Dawson and Ehleringer 1991, Roden and Ehleringer 1999), with a few exceptions such as halophytes (Ellsworth and Williams 2007). Thus the comparison of water extracted from plant stems with likely water sources can identify spatiotemporal patterns of plant water use (Dawson et al. 2002) and water transport (Klaus and McDonnell 2013) in the critical zone.

A quarter century ago, (Dawson and Ehleringer 1991, hereafter DE91) published a landmark paper demonstrating that mature streamside riparian trees in a semiarid, seasonally dry mountain catchment did not use stream water to support transpiration. This surprising conclusion was based on natural variation in the stable isotope composition of hydrogen (δ^2H) in different pools of water. Despite the fact that trees were located within meters of and directly in the stream, DE91 found that mature trees did not use water from the stream. The actual water source was not identified, but available evidence supported the idea that the trees used either groundwater (in subsurface flows below the stream or deeper bedrock layers, with water derived from higher elevation), or perhaps water within the deep soil.

A decade later a series of shallow groundwater wells was installed throughout the semiarid mountain catchment, including the original location of the DE91 study. This opportunity, combined with new advances in stable isotope methods, led us in 2004 to revisit the water sources of the riparian trees in this canyon. Using both hydrogen and oxygen ($\delta^{18}O$) stable isotope ratios, we expected to confirm that the trees of the DE91 study used groundwater, perhaps with seasonal or inter-annual variability in extent of groundwater usage. However, this simple perspective turned out to be incorrect.

Shown in Figure 1 is a dual isotope plot of stream and groundwater, soil water, and water from the stems of four riparian tree species during the summer of 2004 (detail for the methods is provided later). Both stream and groundwater clustered primarily around the local meteoric water line (LMWL), which reflects the well-established dual isotope variation of local rain and snow (Dansgaard 1964). Soil water samples showed enrichment of both of the heavy H and O isotopologues, varying with depth and particularly enriched near the soil surface. Collectively, the soils plotted along a typical slope shallower than the LMWL that indicates fractionation by evaporation (Barnes and Allison 1988). While there was occasional overlap, the water extracted from the stems of the trees was generally below and to the right of the envelope of soil waters, with a shallower slope. Since plant water uptake does not fractionate relative to the water source(s), following the standard interpretation of plant water use studies (e.g., Dawson et al. 2002) one would expect the dual isotope content of stem water to overlap with the water source, or lie between the various possible end members if multiple sources were used. However, the water from the vast majority of stem samples was outside the range of the soils, and deviated markedly in isotope composition from the stream and groundwater, and summer rain (not shown in Fig. 1, but the LMWL is derived from rain and snow). Clearly these healthy trees must have had access to water, but based on the dual isotope data, it seemed that neither summer rain, stream, soil, nor groundwater, nor any combination of them, were supplying transpiration. Nothing like this had been reported at the time, and we were unable to identify the water source.

Several years later, researchers studying a seasonally dry conifer forest in Oregon published a similar result (Brooks et al. 2010). In their case, soils were dry at end of summer, and during the first large fall rainstorm, water infiltrated the soil, adhering tightly to the matric surfaces within the soil. During the storm, the isotope ratios of the rain were altered over time by Rayleigh distillation (Dansgaard 1964). The changing rain from this storm created vertical isotopic variation through the soil profile, with the most

enriched (heaviest) water precipitating from the atmosphere first and adhering to the shallower soil. Water moving (and then tightly adsorbing) deeper in the soil became more and more depleted in heavy isotopes (lighter) as the isotopically changing storm moved over the land surface and infiltration continued. Subsequent rain events during the winter wet season flowed through the soil profile and eventually into groundwater and streams. However, the tightly adsorbed (and isotopically distinct) soil water from the first rain event did not mix with subsequent water, and persisted throughout the next summer to be used by trees when mobile water was absent.

This innovative study and others since have led to the "two water worlds" hypotheses (McDonnell 2014, Evaristo et al. 2015), in which at least two separate water pools exist in the vadose zone; a mobile pool and a second, less-mobile pool that is tightly bound to soil matric surfaces. This idea contrasts with the traditional concept of translatory flow where soil water can be displaced by new water inputs (Buttle 1998), but in reality a continuum of water mobility likely exists in the subsurface. The two water worlds hypothesis involves physical separation of the pools that can be detected by examining soil water, precipitation, and surface/subsurface water in dual isotope space. Both isotopes are needed because the water cycle processes involved lead to spatial separation on such a plot, and thus the possibility of uniquely identifying the pools. The hypothesis does not necessarily include nor rule out isotopic differences between plant stem water and soil water (as in our Fig. 1; see also Fig. 1 of McDonnell 2014), but implies that not all water in soils is equally mobile to transport by hydrologic or biological processes (such as transpiration).

The study of Brooks and colleagues (2010), combined with some understanding of isotope effects associated with a snowpack, led us to investigate the two water worlds hypothesis as an explanation for the unidentified water source of our riparian trees (Fig. 1). The rationale for this is as follows. In much of the mountainous western United States, the soil moisture conditions when winter begins persist under the snowpack for

most of the winter (Maurer and Bowling 2014). If the soil is dry in the fall, that state can continue until late winter when the snowpack melts. Hence the melting of a snowpack infiltrating a dry soil might lead to a unique pool of tightly-bound (less-mobile) water in the soil that differs isotopically from the LMWL. The isotope composition of snow, and particularly snowmelt, could provide a unique isotope signature for the water used by trees the following summer, if the trees make use of the less-mobile water to supply transpiration.

This hypothesis can be illustrated with the conceptual diagrams in Fig. 2. When plotted in dual isotope space, fresh snow is isotopically depleted in both δ^2H and $\delta^{18}O$ relative to rain, but both fall along the same mixing line, defining the LMWL (Figure 2a). A temperate mountain snowpack is subjected to often large temperature gradients (cold and variable near the snow surface, warm and stable near the ground, Burns et al. 2014) and associated vapor pressure gradients that lead to continual vaporization, condensation, and metamorphism (Colbeck 1991, Friedman 1991, Taylor et al. 2001). These phase changes have strong isotopic effects. If there were no loss of water in any phase (equilibrium conditions), then the overall isotope composition of the snowpack as a whole (all phases combined) would remain constant due to mass conservation. However, loss of isotopically light vapor due to ventilation of the snowpack (Bowling and Massman 2011), and loss of liquid during melt lead to marked deviation from the LMWL.

Vapor loss by snow sublimation can be substantial in montane conifer forests (Molotch et al. 2007, Gustafson et al. 2010). Several studies have reported isotopic effects of sublimation (Moser and Stichler 1975, Earman et al. 2006, Sokratov and Golubev 2009, Gustafson et al. 2010) that lead to temporal isotopic changes of the remaining snowpack below and right of the LMWL (Fig. 2b), although such effects are not always observed (Friedman 1991). Additional processes cause fractionation of the snowpack and melt water (Fig. 2c), including melt/refreeze cycles, evaporation from

liquid water, and exchange with atmospheric water vapor (Taylor et al. 2001, Gurney and Lawrence 2004, Zhou et al. 2008, Lee et al. 2010). All of these processes tend to lead the snowpack and melt water derived from it to follow isotopic changes associated with a shallower slope than the LMWL (Fig. 2b, c, Moser and Stichler 1975, Earman et al. 2006, Zhou et al. 2008, Sokratov and Golubev 2009, Lee et al. 2010). These isotopic effects can be imparted to the bulk soil water and to the mobile water phase, and subsequently to streams (Biederman et al. 2014).

Here we investigated winter and spring isotopic effects of the changing snowpack and melt, and whether these isotopic changes could help identify the missing water source for the riparian trees (Fig. 1). We hypothesized that snow sublimation and melt, or similar isotopic effects associated with snowpack metamorphosis, would lead to isotopic enrichment of the snowpack and snowmelt that followed a shallower slope in dual isotope space than the LMWL (Figs. 2b, c). We expected that this water would infiltrate soils during snowmelt, imparting an isotopically-distinct signature to the less-mobile phase in the soil (following the two water worlds hypothesis). We further hypothesized that this less-mobile soil water would be used by the trees to support transpiration during the summer when the soils dried, thus explaining the isotopic enrichment of the water in the tree stems (Fig. 1). These findings would be consistent with snow-derived soil moisture as the primary water source for transpiration of the riparian trees.

Methods

Study location

This study was conducted in riparian mountain meadows of Red Butte Canyon, a protected USDA Forest Service Research Natural Area in the Wasatch Mountains just east of Salt Lake City, Utah. The location is the same as the study of DE91 and some of the same riparian trees were sampled in both studies.

In 2004, two locations were examined, both in riparian tree-meadow ecotones. The first (Brush Basin) was at 1830 m elevation surrounding the main canyon perennial third-order stream, and the second (Todd's Meadow) at 1760 m was bordered by a perennial second-order stream. In 2014, the main focus of the present paper, measurements were conducted only at Todd's Meadow. Sampling strategies and time periods for warm-season sampling were similar during both years. For brevity we provide full methodological details for 2014, and in some cases additional detail for the 2004 season.

The climate at these sites is characterized by hot dry summers and cold winters. Mean annual precipitation of the canyon is elevation dependent, falling primarily as snow in winter, with sporadic summer rain (Ehleringer et al. 1992). Stream flow peaks during snowmelt in March-May and is an order of magnitude greater than in September at base flow. Although 25-30% of annual precipitation falls as rain in the summer, stream flow does not increase during this period (Ehleringer et al. 1992). During the study years of 2004 and 2014, annual peak snow water equivalent at a nearby USDA/NRCS SNOTEL station (Louis Meadow, 2040 m elevation and 5 km distant) was 91% and 61% of the long-term (2000-2015) mean of 657 mm, and total annual precipitation was 99% of the long-term mean (952 mm) in both years.

Alluvial soils at both locations are loamy, well drained, and several meters deep along riparian reaches. The dominant woody flora includes boxelder (*Acer negundo)*, big-tooth maple (*A. grandidentatum)*, water birch (*Betula occidentalis*) and mountain alder (*Alnus incana*) trees that grow along the streams and hillslopes adjacent to the meadows. The meadows are dominated by a variety of grasses and forbs. Further details are available regarding plant community, transpiration and microclimate of Todd's Meadow, including photos (Hultine et al. 2007, Moyes and Bowling 2013, 2015).

Snow and snowmelt sampling

To examine isotopic effects of sublimation and melt we sampled the winter/spring snowpack and melt water. The snowpack was sampled monthly from January 2014 until the snow fully melted in late March 2014 along three distinct 20 m transects. These were representative of snowpack variability within the meadow-forest ecotone: under a sparse tree canopy near the stream, in the open meadow, and under the canopy of a single tree in the meadow. Snowpack cores were collected in 10 cm depth increments from snow surface to soil surface using 5.1 cm diameter polyvinyl chloride (PVC) tubes, and transferred to 0.5 L high-density polyethylene (HDPE) bottles. Bottles were sealed with wax film and allowed to melt before shaking and subsampling into 20 mL glass vials. Vials were sealed with wax film and kept at 4 °C until analysis (same for other water samples described below).

Snowmelt water was collected in three ~19 L plastic buckets, installed before winter in the soil with openings (~63 cm dia.) level with soil surface and below the snowpack. A mesh screen covered the opening to prevent bulk snow or organic litter infiltration throughout the winter prior to melt. A 2.5 cm diameter PVC pipe extended from the bottom of the bucket above the snowpack for sampling. During snowmelt, melted water that collected in the buckets was transferred via tubing and a peristaltic pump through the PVC pipe and collected in 100 mL HDPE bottles. Subsamples were transferred to 20 mL glass vials and stored as above.

Snowmelt water was also collected in the soil using tension lysimeters (Prenart Super Quartz, Denmark). Twelve lysimeters were installed at 35 cm depth into meadow soil prior to winter. Each lysimeter was pumped to 28 kPa below local atmospheric pressure (86 kPa) during sampling to access relatively mobile soil water. Full detail on the lysimeters can be found in Hall et al. (2016a).

Tree xylem water sampling

To examine possible water sources for riparian trees, boxelder and big-tooth

maple trees growing along the bank of the stream were sampled monthly from May until September in 2014. In 2004, two additional tree species were sampled (water birch and mountain alder, all species shown together in Fig. 1). Sampled trees were mature with diameter at breast height > 25 cm, which DE91 found to be important as smaller juvenile trees were shallowly rooted. Sampling dates on May 3, 29, July 1, 28, and September 1 are referred to here as May, June, July, August, and September for convenience. Healthy, suberized distal stems ~ 2 cm dia. were clipped, stripped of bark, phloem, and vascular cambium with a razor blade, and stored as above.

Stream and groundwater sampling

To test for direct uptake of stream or groundwater by trees, we sampled both on each visit. Free-flowing stream water was sampled midstream. Groundwater was sampled from drive-point piezometers (wells) installed in nests at total depths of 9-15 m located within 1-5 m horizontally from tree boles. These were installed using an electric jackhammer and black-iron pipe, as deep as physically possible (until they could be driven no further). The piezometer screens were made of stainless steel (SS) and were ~20 cm in length. HDPE tubing was attached to the screen and extended to the surface such that sampled groundwater was only in contact with SS or HDPE. The piezometers were allowed to fully recover hydraulically between visits. Water levels were measured using an electronic water level meter (102, Solinst, Ontario, Canada) before each purge. Two casing volumes of water were purged prior to sample collection using an inertial pump (404 Solinst, Ontario, Canada) and HDPE tubing. Samples were stored as above.

Soil water sampling

Soil cores were collected to examine tree water usage from within the soil profile, and for comparison to the stream and groundwater in the context of the two water worlds hypothesis. Cores (5 cm dia. x 10 cm long) were taken in 10 cm increments on each visit

to a depth of 1 m from just below the tree canopy and approximately 5 m from the stream bank using a manual ratcheting bucket auger (AMS, American Falls, ID). Contents were emptied into a clean plastic tub homogenized by shaking before a representative subsample was transferred to a glass vial and stored as above.

Additional stem and soil sampling

After finding the pattern in Fig. 1, additional sampling was conducted in Todd's Meadow in summer 2005 to explore deep soil moisture as a possible water source, and to rule out possible sampling artifacts. Soil cores were collected to 2-5 m depth to examine deep soil moisture. Tree stems were sampled as above, then subjected to several treatments a) bark removed as above, b) bark left intact (leaving photosynthetic bark in place), and c) bark-free stems chopped to small pieces in the field and left for ~10 min in the hot sun to maximize evaporative loss before storing in vials. Finally, replicated wood cores were collected from the main bole and a secondary branch using an increment borer (Forestry Suppliers, Inc., Jackson, MS). Soil and xylem samples from these tests were consistent with the results in Fig. 1 (Supplemental Figure 1 and Supplemental Tables 1 and 2).

Water extraction

Water was extracted from stem and soil samples using cryogenic vacuum distillation (Ehleringer and Osmond 1989) and analyzed for isotope composition at the University of Utah's Stable Isotope Ratio Facility for Environmental Research (SIRFER). Samples were completely extracted to ensure quantitative transfer and avoid fractionation (West et al. 2006, Orlowski et al. 2013). Extracted waters were stored with activated charcoal for a minimum of 72 hours to minimize organic compound contamination of water samples prior to analysis. Stream and groundwater were syringe-filtered to 2 μm to remove sediment in water samples prior to analysis. Isotope ratios are expressed relative

to Vienna Standard Mean Ocean Water using standard ‰ notation.

Water samples in 2004 were analyzed by isotope-ratio mass spectrometry (IRMS) for isotope ratios of hydrogen and oxygen as described by Bowen et al. (2005). This method involved high temperature conversion to H_2 and CO gases that were separated using gas chromatography and analyzed by continuous flow IRMS (Delta +XL, ThermoFinnigan, Bremen, Germany).

Water samples in 2014 were analyzed for hydrogen and oxygen isotope ratios using cavity-ringdown spectroscopy (CRDS, L2130-i Analyzer, Picarro, Inc., Santa Clara, CA). We were aware of the possibility that plant secondary compounds in the extracted waters might confound the CRDS data (e.g., West et al. 2010). We performed analyses using both CRDS and IRMS on a subset of the 2014 samples and verified that the CRDS results were not compromised (data not shown). The overall pattern in dual isotope space for all waters was similar in both years (Fig. 1 and Fig. 4, discussed later), providing further confidence that the CRDS data were not altered by organic interference.

Long-term precision for δ^2H and $\delta^{18}O$ of water based on repeated measures of standards at SIRFER for both methods were 1.56 and 0.21 ‰, respectively (Ehleringer et al. 2016).

Electronic soil moisture content and snow depth

Soil moisture was measured in the meadow during 2014 at depths of 5, 10, 20, 50, and 100 cm approximately 10 m from the study trees (Digital TDT Moisture Sensor, Acclima Inc., Meridian, ID). Snow depth was measured using an ultrasonic acoustic depth sensor (Judd Communications, Salt Lake City, UT). These and other meteorological data were obtained from a climate monitoring station in Todd's Meadow associated with the iUTAH EPSCoR project (2014).

Plant water potential

Leaf water potentials were measured at predawn and midday throughout the 2014 season using a Scholander pressure chamber (model 600, PMS Instrument Co., Albany, OR) to assess relative degree of tree water stress.

Statistics

A comparison of slopes of dual isotope plots (δ^2H vs. $\delta^{18}O$) was made for different water pools in 2004 (snow, stem water, soil water, etc.) using analysis of covariance, implemented using the R software package and the lm function (R Core Team 2015).

We further examined distinction between different water pools by testing for multivariate differences between stem water δ^2H and $\delta^{18}O$ and potential water sources using pairwise permutational MANOVA and the Pillai test, implemented with the R package "RVAideMemoire" (Hervé 2016). We analyzed the 2014 data as a whole, and also analyzed each month separately.

Results

The primary melt period was during February and early March, although snow depth increased occasionally with storms into March. Sampling periods for the snowpack and melt water samples are shown relative to snow depth in Fig. 3. Maximum snow depth was ~50 cm. Soil moisture was initially minimal at 50 cm below the surface and deeper, and increased as the snowpack melted (Fig. 3). Soil moisture peaked at all depths at the end of the snowmelt period, and peaked briefly near the surface following occasional rain events throughout the summer and fall. Soil moisture gradually declined at all depths over the summer, and the sampling events for stem, soil, and stream/groundwater captured most of the seasonal variability in soil moisture (Fig. 3).

All snow water samples (snowpack core increments, melt water, and soil lysimeters) plotted along the LMWL or deviated only slightly from it (Fig. 4a, LMWL

coefficients in Table 1, from Hall et al. 2016b). Regression slopes of the samples in dual isotope space were marginally smaller than the slope of the LMWL (7.6) for the snowpack (7.4, Table 1) and lysimeter samples (6.9), but the latter were not statistically different. There was a progressive enrichment in snowmelt isotope composition during the melt period (Fig. 4b) as predicted (Fig. 2c). Snowmelt water collected on March 14 (latest sampling date) strongly influenced the δ^2H vs. $\delta^{18}O$ slope (Fig. 4b). The snowmelt and lysimeter samples prior to March 14 had a slope of 6.8, while the slope of the March 14 samples was 4.2 (Table 1). This progressive melt water enrichment was subsequently manifested in both H and O isotopes of the bulk soil water vertical profile collected after snow melt on March 15 (Figure 4c, only δ^2H shown). The most depleted water (for each isotopologue) was extracted near the soil surface, and the most enriched water was at depth. Deviation from the LMWL was minimal with line-conditioned excess less than -7 ‰ at all depths (Supplemental Figure 2). These data, combined with the soil moisture data (Fig. 3), indicate infiltration of snowmelt water to previously dry soil to at least 100 cm depth. The dual isotope regression slope of this early bulk soil water was 7.2 (r^2=0.97, n=10), similar to the LMWL, but contrasting with our hypothesis of more extreme enrichment associated with sublimation and melt (Fig. 2).

Stream and groundwater samples were indistinguishable and plotted directly along the LMWL, and snowpack and snowmelt samples differed only marginally from it (Fig. 5). In both 2004 (Fig. 1) and 2014 (Fig. 5), water extracted from stems of boxelder and big-tooth maple trees deviated substantially from the LMWL. When each season's samples were considered together, stem water was usually more isotopically enriched in both isotopes than soil water (below and right of soil water, Figs. 1 and 5, Tables 1 and 3). Regression slopes in dual isotope space for soil water in 2004 and 2014 (respectively) were 5.8 and 5.3, and were shallower for stem water, 4.7 and 3.7 (Table 1). We stress, though, we do not intend for a linear regression of stem water samples to be extrapolated back to the LMWL to infer the isotopic composition of source water for the plants. This

is common for either surface water bodies or soils undergoing evaporation, and a similar mechanism occurs in leaf water, but there is no analogous mechanism by which plant xylem water away from the leaves can become similarly enriched.

Stem water measured in 2014 significantly differed in δ^2H and $\delta^{18}O$ space relative to all other measured water pools (Fig. 5) when data were pooled among sampling dates (MANOVA; $p < 0.01$ for each pairwise comparison). Taken as a whole, stem water samples had greater mean $\delta^{18}O$ and δ^2H than the other water pools (Table 3), implying that mean annual stem water could not be explained as a linear mixture of measured source waters.

Examination of stem and soil water on a monthly basis (Fig. 6) with our first statistical test revealed one sampling event when the isotope composition of stem water aligned with the soil evaporation line (July), but in all other months the stem water fell below the soil water isotope line with shallower regression slopes (Table 2). Our second statistical comparison showed similar results (Table 3): stem water δ^2H and $\delta^{18}O$ significantly differed from soil water in May, June, and September (MANOVA $p < 0.01$) but not in July or August ($p = 0.1$ and 0.48, respectively). Leaves on the trees were beginning to emerge during May, and the stem water was highly evaporatively enriched (stems: slope = 3.4, soils: slope = 5.2) with the largest monthly spread of values in dual isotope space (Fig. 6). Once the leaves were fully expanded in June, the isotope composition of stem water was more enriched in dual isotope space than soil water (stems: slope = 3.7, soils: slope = 5.4). In July, the δ^2H vs. $\delta^{18}O$ regression slopes for stem water and soil water were indistinguishable (Table 2), then the slopes diverged again during August - October. (Note that in August our two statistical tests provided diverging results, Tables 2 and 3). There was never a sampling event where stem water matched either stream or groundwater, nor precipitation (which defines the LMWL) in dual isotope composition (Figs. 1, 5, 6). Senescence and leaf abscission occurred in late September.

Despite the soil moisture decrease throughout the growing season (Fig. 3), the pre-dawn water potential of tree xylem remained fairly high (Table 4) with little variability, indicating adequate soil moisture to support transpiration. The most negative midday water potential (-1.74 MPa) was observed in August, indicating only minor water stress for these species (Hacke et al. 2001). During this period, depth to groundwater in Todd's Meadow varied in different wells from 4.5 to 8.6 m below the soil surface, but the water level in each well was highly consistent in depth over time at all sampling visits (Table 5).

Discussion

This study confirms the primary conclusion of Dawson and Ehleringer (1991), yet reveals new complexities of identifying the water sources for these riparian trees. We found, consistently, that these four species of riparian trees do not utilize stream water for transpiration despite their semiarid and generally drought-prone habitat. We note that had we used only hydrogen isotopes (as did DE91 with the methods available then), we would likely have concluded that stream, soil, and groundwater were all possible water sources for the trees. For much of the growing season, stem water samples were often very similar in δ^2H to these pools (Figs. 1, 5, 6). By adding $\delta^{18}O$ and always considering both isotopes together, it is clear that the dual isotope composition of stem water never consistently matched these pools. This underscores recent studies highlighting the combined utility of H and O isotopes in ecohydrological studies (Meißner et al. 2013, McDonnell 2014). Our results are also consistent with the two water worlds hypothesis: stream and groundwater plotted directly along the LMWL, while the water extracted from soils and plant stems deviated substantially from it (Figs. 1, 5, 6), in both years studied.

Here we provide new evidence that these four riparian tree species do not utilize groundwater to support transpiration, which DE91 had speculated was the missing water

source. These data demonstrate that Red Butte Canyon stream and groundwater are not isotopically distinct from local precipitation (Figs. 5, 6), although some of the 2004 data (Fig. 1) deviate a bit more from the LMWL than in 2014 (Fig. 5). If the trees relied on groundwater then water extracted from their stems would overlap groundwater on the dual isotope plots (Figs. 1, 5, 6); this was almost never true. There was spatial variation in groundwater depth within the wells (averaging 4.5 to 8.6 m, Table 5). Considering the spatial locations of the wells, this variability was consistent with distinct and vertically separated layers of groundwater, rather than a steep hydraulic gradient towards or away from the stream. There was no seasonal decrease in groundwater depth as might be expected if transpiration removed substantial groundwater (variability of the water depths across the season in Table 5 was minor), but the pool of available groundwater may be much larger than required for transpiration.

Based on the relatively dry preceding autumn (data not shown), we expected dry soils to persist at depth (>50 cm) through much of the winter, and that deep soil moisture would be recharged during snowmelt (Maurer and Bowling 2014). Shallow soil moisture was recharged in November, but at and below 50 cm, the soils remained dry from late the preceding summer until the snow melted (Fig. 3). The isotopic enrichment of late snowmelt was apparent in soils to 1 m depth immediately after the melt ended (Fig. 4c). Thus one requirement of our hypothesized scenario was satisfied - it is likely that the most tightly-bound soil water at and below 50 cm originated from the snowpack at end of winter. If this water were less mobile than input from later rainstorms during spring and summer (Fig. 3), according to the two water worlds hypothesis the snowmelt water would then persist into the summer rather than being displaced by subsequent storms.

Whereas snowmelt appeared to be an important source of water to deep soils, melt water did not deviate sufficiently from the LMWL to explain trends in stem water isotope composition during the growing season. In contrast to our expectations (Fig. 2b, c), winter and spring snow isotope effects associated with metamorphosis, sublimation, and

melt did not appreciably shift the snowpack or melt water away below and right of the LMWL (Fig. 4). This likely indicates equilibrium and not kinetic isotope effects dominated. We did observe a time-dependent directional enrichment in melt water of δ^2H ~10-15 ‰ (Fig. 4b), but the corresponding change in $\delta^{18}O$ was nearly in equilibrium so the melt water did not deviate strongly from the LMWL (Figs. 4, 5, Supplemental Figure 2). We did not measure sublimation rate, so we are uncertain if sublimation was an important process in this particular winter. Regardless, the minor isotopic change in the 2014 snowpack (Fig. 4) cannot explain the very enriched stem water samples observed the following summer. The stem water samples were fully separated from the snowpack and snowmelt samples on the dual isotope plot (Fig. 5). Thus we reject our overall hypothesis - snowpack isotopic effects were minor, and cannot explain the unique isotopic signature of the xylem water in summer.

Stem water samples in both years were separated in dual isotope space relative to all possible water sources that might support transpiration: precipitation (both rain and snow on the LMWL), stream, soil, and groundwater. Back-diffusion of enriched leaf water (the Peclet effect) can lead to enrichment of xylem in distal organs like the leaf petiole (Barbour 2007), but highly-enriched xylem water was found even in increment cores from the main tree bole (Supplemental Figure 1 and Supplemental Table 2). The stem water enrichment was not caused by evaporation associated with stem photosynthesis, because fairly large stems were used and bark was removed from all stem samples during collection. Alternate collection methods could not explain the high level of enrichment in the stem water (Supplemental Figure 1 and Supplemental Table 2).

While the separation between stem water and other water pools was clear when examining each growing season in total (Figs. 1, 5, Tables 1 and 3), there were important differences within the 2014 season (Fig. 6, Tables 2 and 3). The May field research was conducted just as leaves were beginning to emerge, and the water within the distal stems was quite enriched (Fig. 6). Transpiration rates were not measured, but were probably

small due to low total leaf area and low evaporative demand. It is likely the water in the stems resided there since leaves senesced the preceding autumn, and was enriched due to winter evaporative water loss from the stems. This has been reported previously for big-tooth maple and *Quercus gambelii* (Gambel's oak) in Red Butte Canyon (Phillips and Ehleringer 1995). In that study the stems were measured before and after leaf flush, and once the leaves were fully expanded, the stem waters were much closer to the LMWL. Stem waters in June were closer to the LMWL, but the isotope composition of soil water (and all other water pools) did not overlap with the stem waters during this time (Fig. 6). In midsummer, transpiration rates were high due to a) full canopy leaf area, b) ample soil moisture (Fig. 3), and high evaporative demand (data not shown). Measured pre-dawn and midday water potential (Table 4) indicated that these tree species did not experience significant water stress during 2014 (Hacke et al. 2001, Taneda and Sperry 2008, Hultine et al. 2013). During and only during midsummer, the isotope measurements were consistent with soil moisture as the primary source for transpiration (Figure 6, Tables 2 and 3). In August, and especially September, the dual isotope content of the stem waters again diverged markedly from soil waters (Fig. 6, Tables 2 and 3).

Based on the dual isotope plot, the most plausible water source for our trees appears to be surface soil (0 – 1 m), although soil waters only periodically overlapped stem waters in dual isotope space. Our inability to consistently identify soil water as a tree water source throughout the growing season may be related to additional fractionating mechanisms within the plants or soil, or to the presence of heterogeneous water pools differentially available to plant roots. The mechanism of the enrichment of stem water beyond that of bulk soil water here remains unresolved. However, this phenomenon has been observed in a few other cases (so far without explanation), including the moist temperate conifer forest of Brooks et al. (2010) previously discussed, a semi-arid forest/floodplain ecosystem in Switzerland (Bertrand et al. 2014), a moist subtropical conifer plantation (Yang et al. 2015), and a moist cold Scots pine forest in

Scotland (Geris et al. 2015). There are several possibilities that might explain this pattern.

First, as highlighted by McDonnell (2014), water molecules in the soil system occupy a continuum of water potential, and different methods used to sample soil water likely access it in ways that that may or may not be ecohydrologically realistic (Landon et al. 1999, Zhao et al. 2013, Sprenger et al. 2015, Orlowski et al. 2016). This effect is certain to vary with soil texture since pore size is important for determining matric bonding forces between water and soil particles. Our cryogenic extraction of soil and stem water may not be a realistic substitute for the water potential gradient within the undisturbed soil-root-xylem system, and does not necessarily reflect the isotope composition of water that is actually available for transpiration. We extracted all or nearly all the entire pool of soil water, whereas plants can access only a portion of total soil water. Further, there are very strong matric bonding forces between water and the small diameter xylem cells that may prevent perfect extraction of all stem water via cryogen.

Second, the soils at Todd's Meadow contain a large amount of carbonate, including large (tens of cm diameter) clasts throughout the profiles sampled here. Isotopic interaction of soil water with the carbonate system, perhaps including chemical precipitation and dissolution reactions, is possible and can lead to significant fractionation (Meißner et al. 2013). Third, recent studies highlight the potential for isotopic effects between soil water and cations/clay minerals (Oerter et al. 2014), organic matter (Orlowski et al. 2016, Chen et al. 2016), and rock-water interactions (Lin and Horita 2016, Oshun et al. 2016) that differ for H and O. Finally, we cannot rule out the possibility that our sampling design missed an important pool of bedrock-associated or other groundwater that is the missing source for transpiration of our study trees in Red Butte Canyon. However, given these data we cannot imagine a mechanism whereby that missing groundwater pool could be so enriched to explain the stem water enrichment.

Evaristo and colleagues (2015) have shown that across a wide range of world biomes there is consistent separation between a) precipitation, stream and groundwater, which tend to plot together along the LMWL, and b) soil and plant xylem water, which tend to plot below and right of the LMWL. Our results are in complete agreement. This is evidence that the concept of ecohydrological separation associated with the two water worlds hypothesis is a general phenomenon, and that the water contained in soils, plants and the transpiration flux is distinct from these other pools.

However, a fundamental question remains for the two water worlds hypothesis: How can plants use tightly-bound soil water when more mobile water is available to their roots? The generality observed by Evaristo and colleagues implies that less-mobile water is used by plants most of the time, not only when the mobile water is gone (such as during drought). Water moves through the soil-plant-atmosphere continuum as a continuous liquid column via cohesion and adhesion, along gradients of water potential (free energy). Following mass conservation, the water potential decreases monotonically from the soil end (with least negative water potential) to the leaf end (with most negative water potential) of the column. The driving force for the process is evaporation at the leaf cellular surfaces - no plant-derived energy is required. While plants can regulate hydraulic conductivity of the xylem (Zwieniecki et al. 2001, Javot and Maurel 2002, Boyce et al. 2004) and osmotic potential of living cells, these are minor adjustments to a primarily physical transport system. As a result, even senesced plants and detached roots can transport water (Leffler et al. 2005). Matric forces within the soil are highly dependent on pore diameter due to the surface tension of water films (Nobel 2005), and a substantial energetic requirement must be overcome to remove tightly-bound soil water and use it for plant transpiration. Our leaf water potential data (Table 4) were not negative enough for plants to have been removing tightly-bound water from the soil. Considering the energy gradients involved, it is difficult to imagine a mechanism by which plants could remove and transpire tightly-bound soil water when there is more

mobile water available to their roots; the mobile water would simply follow the energy gradient through the xylem to the atmosphere. Research is needed to help resolve this dilemma.

Conclusions

We found that the dominant riparian trees in our semi-arid ecosystem did not use stream water to support transpiration, supporting previous research at this site (Dawson and Ehleringer, 1991). However, we found no evidence that trees were using groundwater as speculated in the previous study. Trends in $\delta^{18}O$ and $\delta^{2}H$ were most consistent with a surface soil (0 – 1 m) source of water for trees, although stems and soils differed significantly in isotope composition during much of the growing season. This discrepancy points to the potential importance of additional fractionating processes that have received relatively little attention. These include winter evaporative loss, fractionation associated with carbonates or other geochemical and organic constituents, and/or the presence of isotopically distinct water pools accessible at different soil water potentials.

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Table 1. Best-fit slopes and intercepts of regressions between δ^2H and $\delta^{18}O$ for soil waters, stem waters, and snow waters for 2004 and 2014, coefficients of determination (r^2), and sample size (*n*). Standard errors of regression parameters are in parentheses. The LMWL coefficients are shown for comparison (Hall et al. 2016b). Values with different letters are significantly different (comparisons were made among data from a given year only; capital letters for 2004, and lower case for 2014).

	Slope, dimensionless	Intercept, ‰	r^2 *(n)*
2004 Soil Water	5.8 (0.3) A	-35.2 (4.0) A	0.87 *(76)*
2004 Stem Water	4.7 (0.2) B	-58.5 (3.1) B	0.77 *(147)*
2014 Snowpack	7.4 (0.1) a	-1.6 (2.0) a	0.99 *(52)*
2014 Snow Melt	4.8 (0.5) b,c,d	-48.7 (0.5) b,c,d	0.90 *(12)*
2014 Early snowmelt/lysimeter	6.8 (0.2)	-12.2 (3.4)	0.99 (20)
2014 Late snowmelt/lysimeter	4.2 (0.6)	-59.4 (10.8)	0.89 (7)
2014 Lysimeter	6.9 (0.2) a,c	-10.5 (3.0) a,c	0.99 *(19)*
2014 Soil Water	5.3 (0.4) b	-41.7 (3.8) b	0.84 *(81)*
2014 Stem Water	3.7 (0.2) d	-70.9 (1.7) d	0.88 *(77)*
LMWL	7.6 (0.1)	2 (2)	--

Table 2. Monthly linear regression coefficients between δ^2H and $\delta^{18}O$ for stem waters and soil waters during 2014, coefficients of determination (r^2), and sample size (*n*). Standard errors of regression parameters are shown in parentheses. Estimates with different letters are significantly different ($p < 0.05$); lowercase letters denote comparisons among months for stem water, capital letters denote comparisons among months for soil water. *Stem and soil regression lines were statistically equivalent only in July.

Month	Stem water			Soil water		
	Slope, dimensionless	Intercept, ‰	r^2 *(n)*	Slope, dimensionless	Intercept, ‰	r^2 *(n)*
March	3.8 (0.7) a	-66.6 (7.2) a	0.59 (*9*)	7.2 (0.4) A	-10.5 (6.6) A	0.98 (*10*)
April	3.5 (0.5) a	-70.8 (4.5) a	0.92 (*6*)	5.8 (0.5) A,B	-32.6 (8.6) A,B	0.93 (*10*)
May	3.4 (0.3) a	-73.5 (3.2) a	0.93 *(10)*	5.2 (0.4) A,B	-41.2 (5.7) A,B	0.99 *(12)*
June	3.7 (0.4) a	-70.0 (6.1) a	0.89 *(11)*	5.4 (0.2) A	-39.5 (2.7) A	0.99 *(13)*
July*	3.0 (0.8) a	-82.1 (11.2) a	0.64 *(10)*	3.6 (0.5) B	-75.4 (8.6) B	0.79 *(12)*
Aug	5.6 (0.8) a	-47.4 (11.5) a	0.85 *(10)*	3.2 (0.2) B	-80.4 (3.6) B	0.95 *(12)*
Sep	2.9 (1.5) a,b	-83.7 (20.9) a,b	0.30 *(10)*	5.5 (0.3) A	-39.5 (3.5) A	0.98 *(12)*
Oct	1.6 (0.2) b	-97.3 (3.0) b	0.85 (*10*)	6.7 (2.0) A	-15.6 (24.8) A	0.62 (*9*)

Table 3. Mean (standard error) isotopic composition of stem (xylem) water and potential water sources for trees (‰) in 2014 for the full season and for individual months. The means for each isotope significantly differed ($p<0.01$) between stem and soil water in all periods except July and August ($p=0.1$ and 0.48, respectively).

Period	Isotope	Stem	Soil	Stream	Groundwater	Lysimeter	Snow	Snowmelt
Annual	δ^2H	-113.6	-121.2	-122.5	-122	-131.1	-136.9	-132.3
		(1.2)	(1.6)	(0.3)	(0.1)	(2.4)	(2.5)	(1.9)
	$\delta^{18}O$	-11.6	-15	-16.5	-16.4	-17.5	-18.3	-17.5
		(0.3)	(0.3)	(0)	(0)	(0.4)	(0.3)	(0.4)
May	δ^2H	-103.8	-123.2	-122.2	-122.2			
		(3.6)	(1.7)	(0)	(0.2)	--	--	--
	$\delta^{18}O$	-8.9	-15.6	-16.4	-16.4			
		(1)	(0.3)	(0)	(0)	--	--	--
June	δ^2H	-121.4	-122.9	-121.2	-121.5			
		(1.8)	(2.9)	(0)	(0.4)	--	--	--
	$\delta^{18}O$	-13.8	-15.5	-16.3	-16.3			
		(0.5)	(0.5)	(0)	(0.1)	--	--	--
July	δ^2H	-124.4	-127.5	-121.1	-121.9			
		(0.6)	(1.9)	(0)	(0.1)	--	--	--
	$\delta^{18}O$	-14.3	-15.5	-16.3	-16.4			
		(0.1)	(0.5)	(0)	(0)	--	--	--
Aug	δ^2H	-126.3	-129.8	-121.5	-121.7			
		(1.3)	(3.1)	(0)	(0.2)	--	--	--
	$\delta^{18}O$	-14.2	-15.5	-16.3	-16.3			
		(0.2)	(1)	(0)	(0)	--	--	--
Sep	δ^2H	-122.8	-111.2	-123.7	-122.2			
		(1)	(6.4)	(0)	(0.1)	--	--	--
	$\delta^{18}O$	-14.2	-15.5	-16.3	-16.3			
		(0.2)	(1.1)	(0)	(0)	--	--	--

Table 4. Average pre-dawn and midday xylem pressure (MPa) of trees during 2014 (standard deviations shown in parentheses, n=18).

	May	June	July	Aug	Sept
Pre-dawn	-0.78 (0.19)	-0.51 (0.02)	-0.45 (0.04)	-0.38 (0.03)	-0.61 (0.09)
Mid-day	-0.82 (0.19)	-1.6 (0.23)	-1.43 (0.32)	-1.74 (0.23)	-1.49 (0.29)

Table 5. Depths of wells installed among trees and along stream with average depth to top of the water column of each well during 2014 (standard errors shown in parentheses).

	Total well depth (m)	Avg. depth to water (m)
Well 1	11.3	7.0 (0.4)
Well 2	14.9	8.6 (0.3)
Well 3	9.6	4.5 (0.1)
Well 4	14.4	7.5 (0.3)
Well 5	15.2	7.8 (0.2)

Supplemental Table 1. Isotopic composition of water cryogenically extracted from 10-cm sections of three deep soil cores collected in August 2005 at Todd's Meadow. Depths indicated are the center of the 10-cm sections. These are compared to the stem water and LMWL collected in 2004 (repeated from Figure 1) in Supplemental Figure 1.

Core Number	Depth (cm)	δ^2H (‰)	$\delta^{18}O$ (‰)
Core 1	20	-92.9	-11.1
	40	-118.3	-14.7
	60	-118.6	-14.6
	80	-125.6	-15.6
	100	-126.6	-15.7
	120	-129.8	-15.9
	140	-126.7	-16.1
	160	-124.1	-16.2
	180	-123.5	-16.3
	200	-127.1	-16.3
Core 2	20	-103.7	-12.5
	40	-133.7	-16.0
	60	-124.5	-15.8
	80	-132.6	-17.2
	100	-133.7	-16.0
	120	-127.7	-16.7
	140	-125.8	-16.6
	160	-129.3	-16.3
	180	-127.2	-16.7
	200	-123.2	-16.5
	220	-133.7	-16.0
	240	-128.8	-16.4
	260	-124.3	-16.6
	280	-125.7	-16.4
	300	-128.6	-16.6
	320	-126.6	-16.7
	340	-126.4	-16.7
	360	-124.1	-16.3
	380	-125.7	-16.6
	400	-128.2	-16.5
	420	-125.3	-16.5
	440	-121.2	-16.2
	460	-120.0	-15.7
	480	-129.9	-16.6
Core 3	20	-133.7	-16.0
	40	-108.7	-12.9
	60	-114.6	-14.7
	80	-125.0	-15.7
	100	-136.6	-17.2
	120	-127.1	-16.1
	140	-131.5	-16.3

160	-129.9	-17.0
180	-133.7	-16.0
200	-120.6	-15.7

Supplemental Table 2. Isotopic composition of xylem water cryogenically extracted from 3 woody species in August 2005 in Todd's Meadow. Four variations of collection method were used. **Bole sapwood cores** were collected by coring the main stems using an increment borer, and heartwood was discarded. Whole stems (< 2 cm diam.) were collected from distal twigs (with leaves attached) or more proximal to the main stem (with no leaves attached) and left intact without removing bark (**distal stems whole** and **proximal stems whole**, respectively). Distal stems were also collected, bark removed, chopped to pieces in the field, and left to sit in the sun exposed to evaporationb for 10 minutes to examine how hot dry conditions during sampling might influence results (**no bark chopped**). These are compared to the stem water and LMWL collected in 2004 (repeated from Figure 1) in Supplemental Figure 1.

Collection Method	Species	δ^2H (‰)	$\delta^{18}O$ (‰)
bole sapwood core	*Acer grandidentatum*	-135.9	-15.6
		-135.3	-15.5
		-136.6	-15.5
		-133.7	-16.0
		-138.6	-15.1
		-136.1	-15.2
	Acer negundo	-137.8	-15.9
		-133.7	-16.0
		-138.5	-15.6
		-129.8	-14.3
	Betula occidentalis	-135.7	-15.6
		-137.5	-15.7
no bark chopped	*Acer grandidentatum*	-132.3	-15.6
		-133.3	-15.9
		-133.1	-16.0
		-130.4	-15.8
		-133.7	-16.0
		-126.8	-15.6
		-126.7	-15.3
		-130.3	-15.8
		-128.6	-15.6
	Acer negundo	-130.7	-15.7
		-134.1	-16.6
		-129.6	-16.0
		-130.1	-15.7
		-133.6	-15.9
		-133.7	-16.0
	Betula occidentalis	-131.7	-16.0
		-130.9	-16.6
		-129.1	-15.6
proximal stems whole	*Acer grandidentatum*	-135.0	-16.7

		-132.4	-16.4
		-136.7	-17.2
	Acer negundo	-133.7	-16.0
		-135.0	-17.3
	Betula occidentalis	-132.5	-17.3
distal stems whole	*Acer grandidentatum*	-147.1	-19.2
		-130.6	-16.5
		-127.4	-16.3
	Acer negundo	-134.6	-16.9
		-135.7	-16.8
	Betula occidentalis	-135.7	-16.9

Figure Captions

Figure 1: Stable isotope composition of water collected/extracted from soil cores at various depths, stems of trees, adjacent groundwater wells, and nearby streams from June - September 2004, relative to the local meteoric water line (LMWL, Hall et al. 2016b). Tree species included boxelder, big-tooth maple, water birch, and mountain alder in Brush Basin and Todd's Meadow.

Figure 2: Conceptual diagrams of snowpack isotopic change in dual isotope space. (a) Precipitation falling as snow is more depleted than rain, and both fall along and define the LMWL. (b) A snowpack undergoing sublimation vapor loss becomes progressively enriched as the fraction of ice remaining decreases (direction of arrow). (c) The first melt water to leave a snowpack is isotopically depleted, while the snowpack becomes progressively enriched below the LMWL as fraction of ice remaining decreases (direction of arrows). The remaining snowpack can become enriched along the LMWL, and also may progress with a shallower slope. Additional fractionation mechanisms are involved, see text.

Figure 3: Snow depth (top) in 2014, with the time periods of collection of snowpack, snowmelt (bucket) and tension lysimeter samples indicated by horizontal lines. Volumetric water content (bottom) of soil at various depths during 2014. Solid circles correspond to sampling dates for stem, soil, stream, and groundwater during the growing season.

Figure 4: Snowpack and snowmelt isotope composition for winter 2014. (a) Isotope composition of the snowpack in 10 cm increments, snowmelt water collected in buried buckets, and snowmelt water collected with tension lysimeters, relative to the LMWL, (b)

change in snowmelt water isotope composition between Feb. 19, 2014 and March 14, 2014 relative to the LMWL, (c) depth profile of hydrogen isotope composition of cryogenically-extracted soil water at the end of the snow melt period on March 15, 2014.

Figure 5: A dual isotope plot of water isotope composition for 2014, similar to Figure 1 but also including the isotope ratios of the snowpack and snowmelt water. Tree species in 2014 included boxelder and big-tooth maple.

Figure 6: Same as Figure 5 but broken down into monthly sampling events.

Supplemental Figure 1: The LMWL and stem xylem water from Figure 1 compared to water extracted from deep soil cores and from xylem collected using alternative methods. Data are provided in Supplemental Tables 1 and 2, and xylem collection methods are described in the caption of Supplemental Table 2.

Supplemental Figure 2: Depth profile of line-conditioned excess of cryogenically-extracted soil water at the end of the snow melt period on March 15, 2014. The d2H profile for this date is shown in Figure 4c. Line-conditioned excess is similar to deuterium excess but is calculated relative to the LMWL rather than the global MWL (Landwehr and Coplen 2004). Negative values indicate deviation to the right of the LMWL, and zero values would fall along the LMWL.

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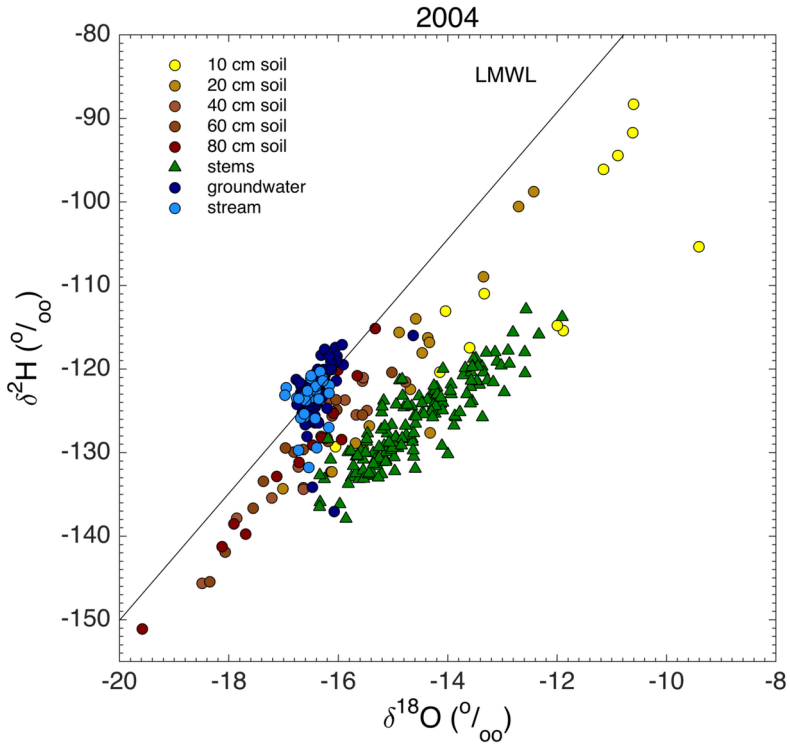

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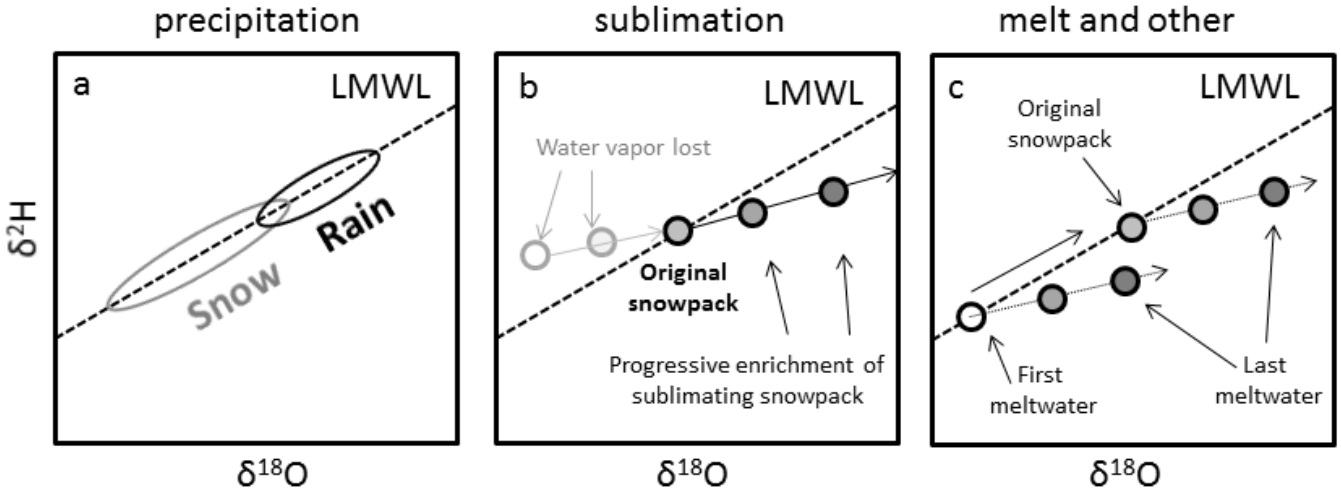

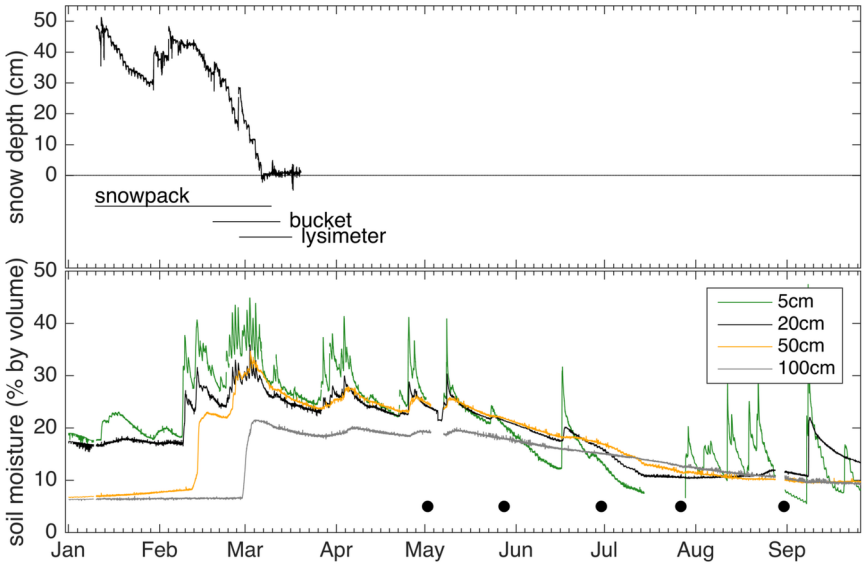

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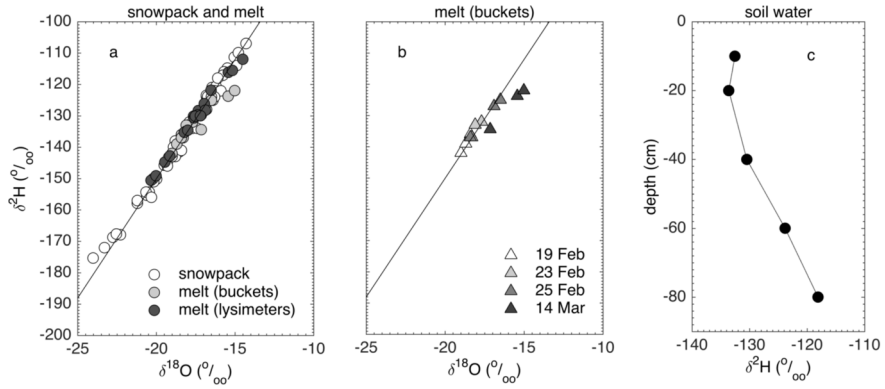

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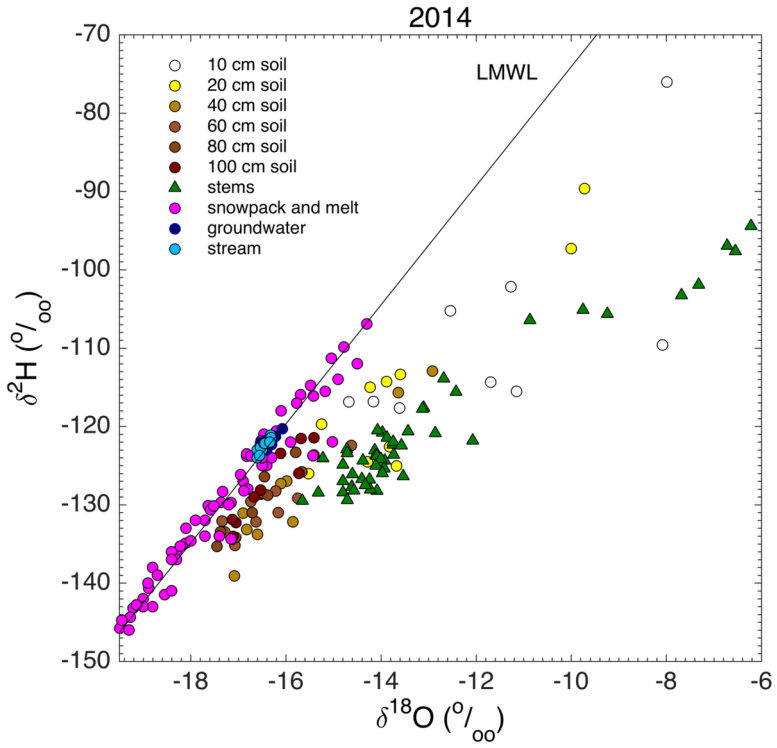

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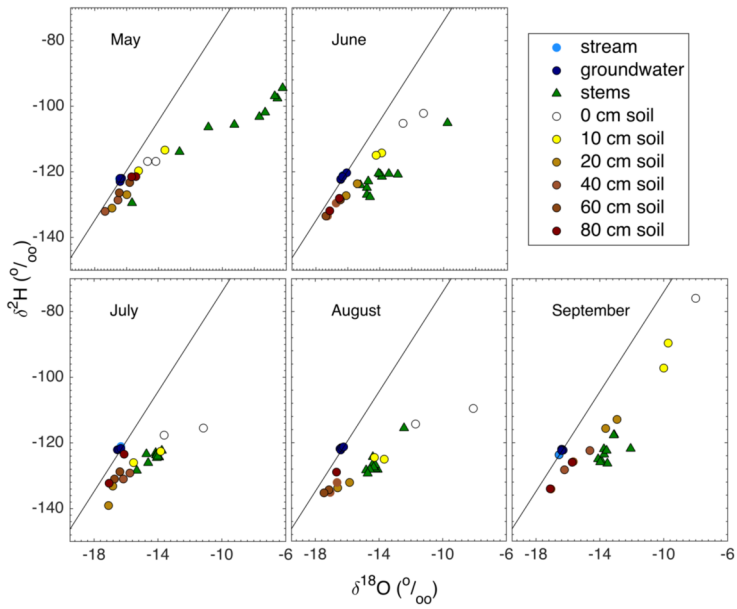

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